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TITLE: Mechanism of Action of Prostate Stem Cell Antigen Targeted Antibody Therapy and Its Relevance to Clinical Application in Prostate Cancer

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14. ABSTRACT We have recently demonstrated that a monoclonal antibody against prostate stem cell antigen (PSCA) can exert anti-tumor activity in a xenograft animal model, suggesting oncogenic activity of PSCA in prostate cancer. Therefore the our goal is to elucidate the role of PSCA in the development of prostate cancer. A better understanding of PSCA function and its antibody activity will enable rational patient selection and trial design, all of which are particularly relevant to subsequent clinical trials of PSCA antibody. There were difficulties in using the LAPC9 xenograft cells to study the effect of suppressing PSCA, but we have since moved ahead to perform an in vivo experiment to study the effect of knocking down PSCA in LAPC9 tumor cells. While the results showed no difference in tumor size, we will repeat this experiment, this time making sure that PSCA is greatly attenuated before innoculating the tumor cells back into mice. The exciting new data came from the in vitro study of overexpressing PSCA in cell lines. We showed that PSCA promote cell growth in prostate cancer cell line 22RV1, but not in normal cells PZ-HPV7. This results agree with previous data that PSCA is upregulated in prostate cancer. We have also engineered the gene targeting construct to make the human PSCA knockin mice, and expect to generate this line in the next 6 month.

15. SUBJECT TERMS
Prostate cancer, prostate stem cell antigen, preclinical mouse model, antibody therapy, antitumor activity

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INTRODUCTION

Previous studies from our laboratory have shown that PSCA is overexpressed in human primary and metastatic prostate cancers. Furthermore, we have recently demonstrated that a monoclonal antibody against PSCA can exert anti-tumor activity in a xenograft animal model, and that this activity was a direct effect mediated by cross-linking. These data suggest that PSCA may have growth-promoting and/or oncogenic activity in prostate cancer. Therefore the our goal is to elucidate the role of PSCA in the development of prostate cancer in order to better understand the mechanism of action of PSCA antibody in tumor inhibition. A better understanding of PSCA function and antibody activity will enable rational patient selection and trial design, all of which are particularly relevant to subsequent clinical trials of PSCA antibody. To accomplish this, we propose to evaluate the requirement of PSCA expression in both xenografts and transgenic models of prostate cancer. We will also generate transgenic models of prostate cancer that express the human PSCA gene to determine the efficacy and toxicity of PSCA antibody therapy.

PROGRESS REPORT

Specific Aim 1. *Investigate the permissive role(s) of PSCA in cancer cell proliferation.*
This aim is being carrying out in parallel to specific aim 2.

Task 1. *Effect of gene silencing of PSCA in cell lines with endogenous PSCA expression.*
Previously we reported that we selected two shRNA constructs (CSCG-GFP-siPSCA) with the most PSCA-knockdown efficiency and subcloned them to generate lentivirus for better infectivity into LAPC9 cells. The backbone of this lentiviral vector also contains GFP to monitor the infectivity and allow isolation of infected cells.

An in vivo pilot experiment was performed using transduced LAPC9 xenograft tumor. Freshly isolated LAPC9 cells were infected with CSCG-GFP-siPSCA or CSCG-GFP alone, and sorted for GFP positive cells after one week. Both control and siPSCA cells were innoculated back into mice and the tumor growth monitored over time. There were no apparent difference in tumor growth or size between both groups. When the tumors were harvested and analyzed for PSCA by FACS, the percentage of PSCA positive cells and the signal intensity in the siPSCA group was comparable to that of control group. This suggested that while the cells used for innoculation was GFP positive, knockdown of PSCA was not effective. We are planning to try using SW780 cells to see if PSCA knockdown has any effect on cell growth.

Task 2. *Effect of overexpressing PSCA in normal prostate epithelial cells (PrECs) and other heterologous cell lines.*
The PZ-HPV7 cell line was transfected with pCDNA3.1 alone or pCDNA-hPSCA and selected under G418 for 2 weeks. These two sublines were compared in terms of growth at different densities. PSCA overexpressing cells appeared to have no growth advantage over control cells at either low or high density. We also overexpressed hPSCA (or the control dsRed, red fluorescent protein) by lentivirus in the prostate cancer cell line 22RV1 and assessed cell growths by MTT assay. In this case, PSCA overexpressing cells exhibited higher growth rate compared to DsRed control at lower density, while at higher density the effect was less prominant (figure 1). This suggests that in prostate cancer cells, PSCA overexpression increases cell growth, while exerts no apparent effect in normal cells.

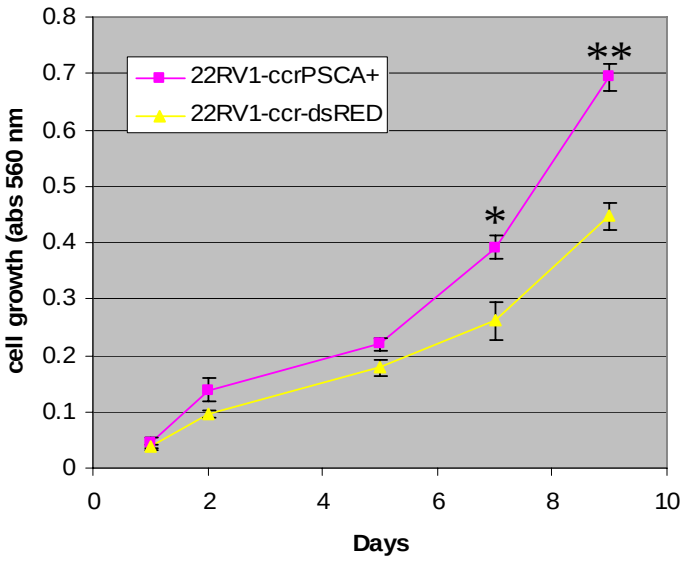

Figure 1. Effects of PSCA on cell growth in 22RV1 prostate cancer cells. *P < 0.011; **P < 0.005

We also seeked to measure anchorage independent growth on soft agar. PSCA-overexpressing 22RV1 or control DsRed cells were seeded at clonal density as single cells in soft agar and maintained for three weeks. Colonies > 300 μm were counted. As shown in figure 2, PSCA overexpression promote anchorage independent growth of 22RV1 on soft agar over DsRed control. To confirm this data, we are generating another PSCA overexpressing 22RV1 subline, as well as testing other cell lines for this growth advantage. Of note, we also test LNCaP and LNCaP-PSCA cells, there was a slight growth increase by cell counting assay (CCk8) in PSCA expressing cells but it was not dramatic, and no difference in anchorage independent growth (figure3).

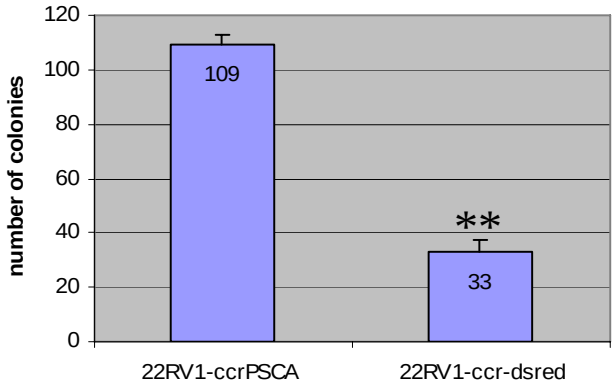

Figure 2. Effects of PSCA on anchorage independent growth in 22RV1 prostate cancer cells. **P < 0.005.

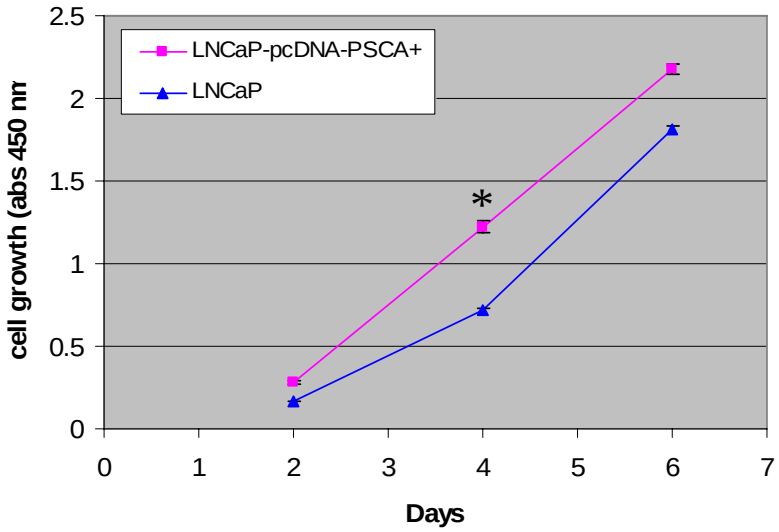

Figure 3. Effects of PSCA on cell growth in LNCaP prostate cancer cells. **P < 0.001.

Specific Aim 2. *Evaluate the role of PSCA in the causation and progression of prostate cancer*.
This aim is being carrying out in parallel to specific aim 1.

Task 1. *Effect of PSCA deficiency in the PTEN$^{-/-}$ transgenic model*.
Due to the difficulty in obtaining the PSCA$^{-/-}$/conditional PTEN$^{-/-}$ compound mice, we were planning to try an alternative approach. This involves obtaining prostate cells from the PSCA knockout mice and infecting the cells with a PTEN knockdown construct, following by combination with the mouse embryonic urogenital sinus mesenchymal cells and engraftment under the kidney capsule of immunodeficient host mice. Such assay has been utilized previously [1] to study murine prostate tissue regeneration, and more recently [2] as a tool to evaluate the result of genetic perturbation in murine prostate cells within 8 weeks. However we encountered difficulties in getting enough mouse prostate cells for infection and for subsequent tissue recombination experiment.

Task 2. *Effect of PSCA deficiency in the Nkx3.1$^{-/-}$ transgenic model*.
We have examined the PSCA$^{-/-}$/Nkx3.1$^{-/-}$ double homozygous compound mice and their Nkx3.1$^{-/-}$ control cohort for difference in the duration to PIN formation in the prostate. The first problem we encountered was that all the mice took much longer time to develop prostatic intra-epithelial neoplasia (PIN), even in the Nkx3.1$^{-/-}$ control group. The most obvious abnormalities was observed in the anterior prostates at 16 weeks, and here there was no difference between the double knockout group and the control group. Following this time point, we have acquired tissues from mice groups at 32 weeks and one year for histology assessment, and found no clear difference between the double homozygous compound mice and their Nkx3.1-/- control group. Therefore it appears that in this model, PSCA deficiency did not accelerate or delay PIN formation.

Specific Aim 3. *Assess the efficacy and physiological effects of the antibody in a preclinical model expressing human PSCA.*

Task 1. *Development of transgenic model of prostate cancer expressing human PSCA.*
We have since revised our strategies for developing this preclinical model. Initially we proposed to generate a transgenic mouse overexpressing human (h)PSCA using the human PSCA promoter while its mouse counterpart is not disturbed, and subsequently cross it with PTEN null mice. However, we did acknowledge the concern that targeting human PSCA alone may not be sufficient to inhibit tumor growth since the expression of mouse PSCA is not targeted. Therefore, our current approach is to specifically place the human PSCA cDNA under the mouse promoter by "knock-in" gene targeting, thus effectively silencing the mouse PSCA by expressing its human counterpart.

The construct for gene targeting was made in the past year. We screened the CITB mouse BAC library by oligonucleotide probe, and three clones were identified. Upon further confirmation by PCR using primers specific for mouse PSCA, only one BAC clone was positive. A EcoRI/XhoI 9.8 kb fragment containing mPSCA gene was isolated and subcloned into Bluescript plasmid. From here, a 5' and 3' genomic arms were generated by restriction digest and PCR cloning. The 5' arm was 3 kb, starting from the transcriptional start site going upstream, while the 3' arm started within the first intron of mPSCA and ended at XhoI site at the 3' end of the gene. Thus the first exon and first half of the intron in mPSCA gene will be targeted for deletion and replaced with human PSCA cDNA. The genomic arms were strategically subcloned into a gene targeting vector, pGKneo-F2L2DTA (figure 4). Once the sequence of this vector was confirmed, it will be transfected into ES cells to select for neomycin resistant positive clone, and subsequently to generate the hPSCA knockin mouse.

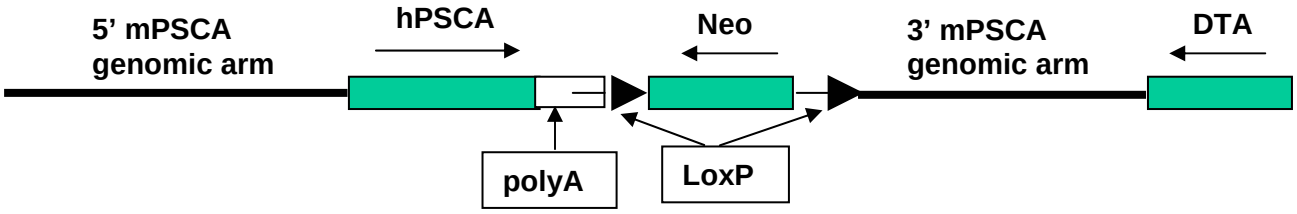

Figure 4. Shematic representation of gene targeting construct of hPSCA. Neo – neomycin resistance, for selection of recombinant ES clone; DTA – diptheria toxin A, selecting against random integration; LoxP – site for Cre recombinase to remove neomycin cassette.

Task 2. *Studies of PSCA antibodies in transgenic models.*
This task will be performed once the preclinical model is established.

KEY RESEARCH ACCOMPLISHMENTS

- In vivo experiment of LAPC9 xenograft tumor cells.
- Overexpression of PSCA promotes cell growth in 22RV1 cells, but not PZ-HPV7 cells, in vitro.

- Examine PSCA$^{-/-}$/Nkx3.1$^{-/-}$ double knockout mice at later time point and find no difference in the extent of time taken to PIN formation compared to control group.
- Making the preclinical model by using gene targeting “knock-in” technology.

REPORTABLE OUTCOMES

None

CONCLUSION

We have encountered difficulties in using the LAPC9 xenograft cells to study the effect of suppressing PSCA, but we have worked out conditions to overcome this problem and moved ahead to perform an in vivo experiment to study the effect of knocking down PSCA in LAPC9 tumor cells. While the results showed no difference in tumor size, we will repeat this experiment, this time making sure that PSCA is greatly attenuated before innoculating the tumor cells back into mice. The exciting new data came from the in vitro study of overexpressing PSCA in cell lines. We showed that PSCA promote cell growth in prostate cancer cell line 22RV1, but not in normal cells PZ-HPV7. This results agree with previous data that PSCA is upregulated in prostate cancer.

Although the PSCA$^{-/-}$/Nkx3.1$^{-/-}$ double knockout mice that we generated did not show any difference in the time duration to PIN formation in comparison to control group, this does not rule out the role of PSCA in prostate cancer. It is possible that PSCA is more important in the progress to metastatic cancer than in the primary tumor stage, since we have shown that PSCA protein and mRNA are highly elevated in clinical specimen of prostate cancer metastases [3]. We have also engineered the gene targeting construct to make the human PSCA knockin mice, and expect to generate this line in the next 6 month.

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